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(54) PROCESS AND APPARATUS FOR INCREASING THE PERMEABILITY OF THE MEMBRANE OF CELLS OF ORGANISMS

(71) We, KERNFORSCHUNGSANLAGE JÜLICH GESELLSCHAFT MIT BESCHRÄNKTER HAFTUNG, of Postfach 365, 517 Jülich, Federal Republic of Germany, a Body Corporate organised according to the laws of the Federal Republic of Germany, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The invention relates to a process for increasing the permeability of the membrane of cells of organisms to apparatus for the execution of the process, to cells treated by the process, and to the use of such cells.

In accordance with an as yet unpublished proposal, cells of living species are added to a solution containing complex forming substances or substances with a catalytic activity, at a lower osmolarity compared to that of the cell contents. Owing to the resulting increased permeability of the cell membrane an exchange of substance occurs during this operation between the solution contained within the interior of the cells and the solution containing complex forming substances or substances with a catalytic activity. Thereafter, the osmolarity of the solution containing the cells is increased to the osmolarity of the cell content of the initially introduced cells, by the addition of substances with an osmotic activity, such as calcium, potassium and sodium ions, whereupon the cell membrane loses its permeability for the complex forming substances or substances with a catalytic activity contained within the interior of the cell, so that they become occluded therein.

Cells treated in this manner and containing complex forming substances can be employed for the separation of ionised compounds from an aqueous solution. For this purpose, the cells containing the complex forming substances can be introduced into the aqueous solution containing the ionised compounds so that the ionised compounds migrate through the cell membrane, and are converted by the complex forming substances to complexes of low degrees of dissociation or of low solubility. When the cells are separated from the aqueous solution, the ionised compounds combined within the cells are also separated from the aqueous solution.

Cells containing substances with a catalytic activity can be employed for the synthesis or degradation of substances contained in an aqueous solution. For this purpose, the cells can be applied in the aqueous solution until the substances to be synthesised or degraded and contained in the aqueous solution have migrated into the interior of the cells due to the permeability of the membrane of the cells, the synthesis or the degradation of the substances are ended, and the substances have migrated into the aqueous solution through the membrane of the cells, whereupon the synthesised or degraded substances can be separated from the aqueous solution.

The proposed process step causing the increase in the permeability, in which the cells are introduced into a solution having a lower osmolarity compared to the osmolarity of the cell contents, does however take a lot of time, since the increase in permeability only takes place slowly, and since in addition attention must be paid to several parameters of importance in the process step. Moreover, in the case where bacterial cells are employed as the cells and where it is necessary to remove the cell wall, an additional process step has to be applied in order to separate the cell wall.

There is thus a need for a process for increasing the permeability of the membrane of cells of organisms which may

be simply, quickly and hence economically carried out.

According to the invention there is provided a process for increasing the permeability of the membrane of cells of organisms, wherein a physiological electrolyte solution containing the cells dispersed in suspension therein is subjected to an electrical field.

Preferably the electrolyte solution containing the cells in suspension has a temperature in the range 0°C to 25°C when subjected to the electrical field.

Suitably the electrical field is applied until macromolecules having a radius of at least 5 Å are exchanged through the cell membrane between the solution contained in the interior of the cell and the physiological electrolyte solution. For this purpose, the field strength of the field providing the increase in permeability is appropriately of 10^3 to 10^5 V/cm. A high rate of exchange of macromolecules having a radius of at least 5 Å can be achieved.

The process of the invention may be discontinuously or continuously executed. In order to carry out a discontinuous process, a container, in which two electrodes are arranged, may be filled with a physiological electrolyte solution containing the cells of organisms in suspension, and an electrical impulse is applied to the electrolyte solution. For the continuous execution of the process of the invention, the physiological electrolyte solution containing the cells in suspension may be passed through the electrical field in a container filled with physiological electrolyte solution, a constant electrical field being applied by electrodes therein. This is advantageously carried out by supplying fresh physiological electrolyte solution containing the cells dispersed in suspension therein continuously to the container and at the same time sucking from the container electrolyte solution containing cells which have been subjected to the electrical field. This mode of operation also discharges the heat formed in the electrolyte solution by the electrical field. Advantageously the physiological electrolyte solution containing cells in suspension is passed through the focus of a focussed electrical field. This achieves a better exploitation of the electrical field and at the same time ensures that all of the cells led through the electrical field are exposed to a nearly constant field strength.

It has been found that the contact time of the cells in the electrical field causing the increase in the permeability, which is required for increasing the permeability of the cell membrane may be very short so that cells with an increased permeability of the cell membrane can be simply and rapidly produced, and also at a high yield.

Particularly advantageously the physiological electrolyte solution containing the cells is passed through an aperture in a wall made from electrically non-conducting material, arranged between the electrodes for the electrical field, so that the aperture surrounds the focus of the electrical field. This achieves an even better exploitation of the electrical field, all the cells being being subjected to a practically constant electrical field. At the same time, an even more complete exchange of macromolecules through the cell membrane can be achieved. This can for instance be recognised during the use of erythrocytes by the discolouration of the electrolyte liquid due to the haemoglobin emerging from the interior of the cell, and by the discolouration of the erythrocytes.

According to the invention in another aspect there is provided apparatus for carrying out the process of the invention as described above, having two chambers to contain electrolyte solution separated by a wall of electrically non-conducting material, a passage through the wall, and an electrode in each chamber, the arrangement of the electrodes and passage being such that an electrical field between the electrodes is focussed at the said passage, there further being an inlet into one chamber for the electrolyte solution containing the cells and an outlet from the other chamber for the solution containing the cells which has passed through the said passage. The separating wall between the chambers may for example be of glass. In processes embodying the invention, the diameter and the length of the passage, and the electrical field applied to the electrodes, should be dimensioned in accordance with the required throughput rate so that the intended increase in the permeability of the cell skin is achieved.

Preferably the said passage has a minimum cross-sectional dimension of at least 20 μm. Advantageously, for topping up the level of electrolyte the apparatus has a second inlet for supply of electrolyte solution separate from the said inlet for the electrolyte solution containing the cells.

Preferably the said inlet for the electrolyte solution containing the cells is a nozzle directed towards the said passage. Also the said outlet for the solution containing cells which has passed through the passage may be a discharge pipe whose entrance is directed towards the said passage. Desirably the solution containing treated cells is extracted from the chamber through the outlet by suction.

It has been found that the increase in the permeability of the membrane of cells can

be reversed i.e. their normal permeability restored within 1 to 2 hours by heating the solution containing the cells to a temperature lying between 15 and 40°C. When bacterial cells are employed, the cells are for this purpose appropriately heated to a temperature of 20°C, and when erythrocytes are employed, these are preferably heated to a temperature of about 37°C. The fact that the normal permeability of the cell membrane can be recreated in this manner renders the cells suitable for the incorporation of macromolecules of widely varying types and hence for a variety of different use.

The invention therefore embraces a process for the use of cells having their permeability increased by a process of the invention, wherein the cells are exposed to a solution of a complex forming or catalytically active substance to be occluded in the cells, the temperature is raised, and thereafter the cells are exposed to a solution containing ions to be complexed or one or more reactants to undergo reaction in the presence of the catalytically active substance.

The cells of organisms treated by a process of the invention and having a complex forming substance occluded therein can advantageously be used in processes for the separation of ionised compounds, such as heavy metal ions, from a mixture of compounds dissolved in an aqueous solution containing at least 0.5 mM of magnesium and/or calcium ions as well as potassium ions, such as sea water, fresh water, or waste water, by means of organic or inorganic complex forming substances which promote the separation and enter into combination with the compounds to be separated. For this purpose, the cells may be introduced to a solution containing the complex forming substances, the osmolarity of which differs at most within limits from the osmolarity of the cell contents of the original cells and from the osmolarity of the aqueous solution, for such a time until the cell contents are practically the same as the solution containing the complex forming substances due to exchange of material through the cell membrane until a state of equilibrium has been attained between the solution contained within the interior of the cell and the solution containing the complex forming substances. In order to restore the normal permeability, the solution containing the cells is thereafter kept at a temperature in the range of 15 to 40°C for 1 to 2 hours after being heated up. Subsequently, the cells containing the complex forming substance are separated from the solution containing the complex forming substances. In order to concentrate the ionised compounds contained in the aqueous solution, the cells are then inserted in the aqueous solution until the ionising compounds to be separated from the aqueous solution have migrated through the cell membrane into the interior of the cells, and have been converted by the complex forming substances to complexes with a low degree of dissociation or at a low solubility. The cells can then be separated from the aqueous solution.

The cells of organisms treated by a process embodying the invention are also suitable for use in processes for the synthesis and degradation of substances and dissolved in an aqueous solution containing at least 0.5 mM of magnesium and/or calcium as well as potassium ions, by means of materials with a catalytic activity which promote synthesis or degradation. For this purpose, the cells are held in a solution containing catalytically active materials, the osmolarity of which differs at most within limits from the osmolarity of the cell contents of the original cells and from the osmolarity of the aqueous solution, for such a time until the cell contents are practically the same as the solution containing the catalytically active materials, due to the exchange of material through the cell membrane with its increased permeability, between the solution contained within the interior of the cell and the solution containing the catalytically active materials. Thereafter, the solution containing the cells is kept at a temperature in the range of 15 to 40°C for 1 to 2 hours after being heated up. Subsequently, the cells containing the catalytically active materials are separated from the solution containing the catalytically active materials. In order to execute the process for the synthesis or degradation of substances, the cells are then applied in the aqueous solution until the substances to be subjected to synthesis or degradation, which are contained in the aqueous solution, have migrated through the membrane of the cells into the interior of the cells, the synthesis or degradation of the substances has been completed, and the substances have migrated into the aqueous solution through the membrane of the cells. The substances thus formed by synthesis or by degradation can then be separated from the aqueous solution.

Two embodiments of the invention will now be described by way of example with reference to the accompanying drawings, in which:—

Figure 1 illustrates apparatus embodying the invention, having a container subdivided into two chambers by a dividing wall, and

Figure 2 illustrates another apparatus

embodying the invention, having three containers arranged concentrically.

Referring to Figure 1 a container 1 is subdivided into chambers 4, 5 by a dividing wall 2 of non-conducting material, which has in it a passage 3. An electrode 6 is provided in each of the chambers 4, 5. Pipe connections 7 are provided on the container wall for the separate passage of physiological electrolyte solution into chambers 4, 5. To carry out a process embodying the invention, the physiological electrolyte solution containing the cells is supplied to the chamber 4 via a supply nozzle 8 which is directed towards and opens in the vicinity of the passage 3, and is sucked again from the container 1 via discharge pipe 9 having passed through the passage 3 which surrounds the focus of the electrical field, to be captured in a cooled receiver arranged in front of the suction pump and not illustrated in Figure 1. The entrance to the pipe 9 is also directed towards and is in the vicinity of the passage 3. Losses of physiological electrolyte solution are compensated for via the supply pipes 7.

Referring now to Figure 2, three containers 10, 11, 12, of non-conducting material have a concentric arrangement within each other, so as to form an outer, an intermediate and an inner chamber. Concentric electrodes 13, 14 are respectively arranged in the outer and the intermediate chambers and leads for these chambers pass through the walls of the containers. The outer container provides a chamber 4 and the intermediate chamber a container 5 equivalent to the chambers 4 and 5 of Figure 1. The lower part of the intermediate chamber provides a dividing wall between them. In order to carry out a process embodying the invention, the electrolyte solution containing the cells is supplied to the outer chamber via an inlet nozzle 15, (corresponding to the inlet 8 of Figure 1) and passes through a passage 16 surrounding the focus of the electrical field and then through an aperture 17 into the inner container 12. This is effected by the suction of electrolyte solution via a pipe connector 18 opening into the container 12 (which acts as the outlet from the chamber 5). The passage 16 corresponds to the passage 3 of Figure 1, and has a minimum cross-sectional dimensions, namely to a diameter, of at least 20 μm. The cells removed by suction are captured in a cooled receiver arranged in front of the suction pump and not illustrated in Figure 2. The losses of physiological electrolyte solution incurred during the execution of the process in the device are compensated for via the pipe connections 19 and 20. Pipe connector 21 merely serves for the removal of air from the apparatus. A thermocouple 22 or other temperature sensor is provided for the control of temperature in the inner chamber in order to make it possible to eliminate the possibility of excessive heating and hence damage to the cells.

An example of a process embodying the invention will now be described.

Example

About 100 ml of fresh cattle blood was collected in an isotonic sodium citrate solution, and the resultant solution centrifuged off in a centrifuge at 1200 g. Subsequently, about 30 ml of the concentrated erythrocytes which had been centrifuged off were washed twice with 100 ml of a buffer solution containing 150 mM NaCl, 16 mM KCl, 4 mM $MgCl_2$, 2 mM $CaCl_2$ and 5 mM Tris per litre, the pH value of which had been adjusted to 7.4 by the addition of hydrochloric acid, the cells being centrifuged off each time. "Tris" is tris (hydroxymethyl) aminomethane. Subsequently, the erythrocyte concentrate was diluted with buffer solution to which 1 mM adenosine triphosphate per litre had been added, in the ratio of 10:3.

Thereafter, the solution containing the erythrocytes was sucked through the supply nozzle 15 of the apparatus illustrated in Figure 2, to which buffer solution serving as the physiological electrolyte solution and cooled to 0°C was supplied at the same time. The diameter and the length of the passage 16 provided in the intermediate container 11, as well as the distance between the tip of the supply nozzle 15 and the passage 16, was 0.45 mm. A potential of 350V was applied to the electrodes. The throughput rate of the erythrocytes through the device was adjusted so that the amount of erythrocytes introduced had passed through the device within about 30 minutes. The erythrocytes collected in the receiver were centrifuged off at 0°C and 13,000 g for about 15 minutes.

Subsequently, 0.5 ml of the erythrocytes which had been centrifuged off were suspended in a solution made up of 5 ml of buffer solution and 0.2 ml of an iodine-131/albumen solution, the specific activity of which was 0.1 mCi/ml, and kept at 0°C for about one hour. The solution was thereafter warmed, and kept at 37°C for about two hours. Subsequently, the erythrocytes were centrifuged off at 13,000 g for 15 minutes, and the centrifuged-off erythrocytes were washed twice with buffer solution containing 0.1% of albumen as the carrier, being centrifuged off each time. The activity of the iodine-131 remaining in the erythrocytes was measured after hydrolysis of the

erythrocytes in a TriCarb Liquid Scintillator. ("TriCarb" is a Registered Trade Mark). The activity measured corresponded to a 31% absorption of iodine-131 by the erythrocytes from the solution containing iodine-131/albumen.

WHAT WE CLAIM IS:—

1. A process for increasing the permeability of the membrane of cells of organisms, wherein a physiological electrolyte solution containing the cells dispersed in suspension therein is subjected to an electrical field.

2. A process according to claim 1 wherein the electrolyte solution containing the cells in suspension is at a temperature in the range 0° to 25°C when subjected to the electrical field.

3. A process according to claim 1 or claim 2 wherein the electrical field is applied until macromolecules having a radius of at least 5 Å are exchanged through the membranes of the cells between the solution contained in the interior of the cell and the physiological electrolyte solution.

4. A process according to any one of claims 1 to 3 wherein the physiological electrolyte solution containing the cells is passed through the focus of a focussed electrical field.

5. A process according to claim 4 wherein the physiological electrolyte solution containing the cells is passed through an aperture surrounding the focus of the electrical field in a wall made of an electrically non-conducting material and arranged between electrodes providing the electrical field.

6. A process for increasing the permeability of the membrane of cells of organisms by the use of an electrical field substantially as herein described with reference to Figure 1 or Figure 2 of the accompanying drawings or in the Example.

7. Apparatus for carrying out the process of any one of the preceding claims having two chamber to contain electrolyte solution separated by a wall of electrically non-conducting material, a passage through the wall, and an electrode in each chamber, the arrangement of the electrodes and passage being such that an electrical field between the electrodes is focussed at the said passage, there further being an inlet into one chamber for the electrolyte solution containing the cells and an outlet from the other chamber for the solution containing the cells which has passed through the said passage.

8. Apparatus according to claim 7 wherein the said passage has a minimum cross-sectional dimension of at least 20 μm.

9. Apparatus according to claim 7 or claim 8 having a second inlet for supply of electrolyte solution separate from the said inlet for the electrolyte solution containing the cells.

10. Apparatus according to any one of claims 7 to 9 wherein the said inlet for the electrolyte solution containing the cells is a nozzle directed towards the said passage.

11. Apparatus according to any one of claims 7 to 10 wherein the said outlet for the solution containing the cells which has passed through the passage is a discharge pipe whose entrance is directed towards the said passage.

12. Apparatus according to any one of claims 7 to 11 including means for extracting the solution containing the cells from the chamber through the said outlet by suction.

13. Apparatus according to claim 7 wherein the two chambers and the outlet for the solution containing the cells are provided by the outer, intermediate and inner of three containers made of electrically non-conducting material and arranged concentrically one within another, the outer and intermediate containers each having within them an electrode, the two electrodes being concentric, an inlet for the supply of the electrolyte solution containing the cells extending into the outer container through the centre of its bottom, the intermediate container having the said passage extending through its bottom opposite the said inlet, the passage having a minimum cross sectional dimension of at least 20 μm, and the bottom of the inner container having an aperture opposite to the said inlet and to the said passage; the outer container and the upper part of the intermediate container also having respective pipe connections for the supply of electrolyte solution and the upper part of the inner container having a pipe connection for the removal by suction of the electrolyte solution containing the cells.

14. Apparatus according to claim 13 wherein the inner container has a duct for a thermocouple or other temperature sensing element.

15. Apparatus according to claim 13 or claim 14 wherein the outer chamber also has a pipe connection for the removal of air.

16. Apparatus for carrying out the process of claim 1 substantially as herein described with reference to and as shown in Figure 1 or Figure 2 of the accompanying drawings.

17. Cells treated by a process according to any one of claims 1 to 6.

18. A process for the use of cells having

their permeability increased by the process of any one of claims 1 to 6 wherein the cells are exposed to a solution of a complex forming or catalytically active substance to be occluded in the cells, the temperature is raised, and thereafter the cells are exposed to a solution containing ions to be complexed or one or more reactants to undergo reaction in the presence of the catalytically active substance.

MEWBURN ELLIS & CO.,
Chartered Patent Agents,
70—72, Chancery Lane,
London, WC2A 1AD.
Agents for the Applicants.

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1481480 COMPLETE SPECIFICATION

2 SHEETS *This drawing is a reproduction of the Original on a reduced scale*

Sheet 1

FIG.1